27 cont'd

claim 23 under conditions suitable for expression, processing and secretion of said sIL-6R/IL-6 into the culture medium in which said cells are grown; and purifying said sIL-6R/IL-6 from said culture medium.

Please delete claims 27-32 without prejudice.

Please amend claim 33 as indicated.

33

33 (Amended). A pharmaceutical composition comprising as active ingredient a chimeric sIL-6R/IL-6 according to claim 38, and a pharmaceutically acceptable carrier, diluent or excipient.

Please delete claims 34-36.

REMARKS

Claims 2-7, 9-11, 16-26, 33, 37 and 38 presently appear in this case. No claims have been allowed. The official action of January 15, 2002, has now been carefully studied. Reconsideration and allowance is hereby respectfully urged.

Briefly the present invention relates to chimeric polypeptides constructed from the fusion of the naturally occurring form of soluble IL-6 receptor (sIL-6R) and IL-6 as well as DNA encoding same, vectors made from said DNA, and methods of making. The present invention is also related to

pharmaceutical compositions containing such polypeptides and methods of use for the treatment of cancer and liver disorders, enhancement of bone marrow transplantation, and treatment of other IL-6 related conditions.

The examiner has retained the restriction requirement on the ground that the Fischer reference anticipates the only technical feature common to the present claims and thus there is no common inventive concept for the present claims. This restriction requirement is again respectfully traversed.

The claims have now been amended in a manner so as to clearly and definitely avoid anticipation by Fischer as will be discussed below in greater detail with respect to the anticipation rejection. As all of the claims now ultimately depend from claim 38, they all share the same common technical feature. Accordingly, at this point, reconsideration and withdrawal of the restriction requirement is in order.

The examiner has objected to the drawings because Figure 1(c) has not been described in the brief description of the drawings.

The description of Figure 1 has now been amended to include reference to Figure 1(c) thus obviating this objection.

The examiner has objected to the amendment filed July 24, 2001, as introducing new matter into the disclosure. The examiner has required cancellation of the indicated new matter.

The paragraph of the specification in question has now been further revised in order to delete all of the language which the examiner considers to be new matter, and to refer more specifically to the exact wording in the Figure which precedes the sequence whose sequence is being designated. Accordingly, as this sentence refers only to language explicitly appearing in the figures, no new matter is present, thus obviating this objection.

Claims 1-5 and 32-36 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite. The examiner states that claim 1 is indefinite, as are the terms "being glycosylated in a similar fashion to the glycosylation of naturally-occurring sIL-6R and IL-6", "essentially all", "analog" and "biological activity".

Claim 1 has now been amended so as to avoid use of the terminology noted by the examiner except that "analog" still remains in the claim but has been defined in the manner in which it was defined in the specification at page 18, paragraph beginning at line 4. Accordingly, this rejection of claim 1 has now been obviated.

Claims 2-5, 13, 14, 15, and 32-33, have been rejected as being indefinite for the use of "biologically active analogs" and "analogs" for the reasons given in the rejection of claim 1.

These claims have also now been amended so as to delete use of the terminology objected to by the examiner, except possibly to refer back to the same language which has been appropriately defined in claim 1. Accordingly, this part of the rejection has also been obviated.

The examiner states that claims 6 and 7 are indefinite because the SEQ ID NO. of the sequence containing Val 326 and Pro 29 are not disclosed. The examiner states that "positions 357-359" refer to amino acids of a polypeptide which has not received a SEQ ID NO. and the same is true with respect to Met 212 and Val 112 in claim 8. In claims 6 and 7, the examiner considers the term "sIL-6RδVal/IL-6" as not being an art accepted term without a corresponding SEQ ID NO.

Claim 6 has now been amended to recite the SEQ ID NO. of the full sequence and to delete reference to Val-356 and Pro-29. Claim 7 has been amended to refer to the SEQ ID NO. of all parts of the sequence, and to delete reference to the Val-356 and Pro-29. Claim 8 has been deleted. Accordingly, this rejection has now been aviated.

The examiner considers claim 9 to be indefinite in use of the terminology "in a fully processed form".

This terminology has now been deleted from claim 9, thus obviating this part of the rejection.

The examiner indicates indefiniteness in claims 12-15 and 32. These claims have now been deleted without prejudice, thus obviating this part of the rejection.

Accordingly, reconsideration and withdrawal of the entire rejection under 35 U.S.C. 112, second paragraph, is respectfully urged.

Claims 1-15 and 32-36 have been rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the chimera disclosed in SEQ ID NO:7, does not reasonably provide enablement for analogs thereof with no defined structure. The examiner states that there is lack of guidance as to what minimal structure requirements are necessary for a functional chimeric protein. This rejection is respectfully traversed.

New claim 38 now specifically recites the definition of analog as appearing in the specification in the paragraph beginning at page 18, line 4, and the paragraph beginning at page 19, line 3. Accordingly, claim 38(b) specifies that the analog differs from the sequence of (a) by no more than 30 changes in the amino acid sequence of (a), each such change

being a substitution, deletion, or insertion of a single amino acid, which analog retains the capability of triggering the dimerization of gp130 in human cells. As there are over 500 residues in the fusion product of (a), the recitation of up to 30 modifications therein results in a maximum of less than 6% changes, i.e., there will always be at least 94% identity among the analogs of claim 38(b). Furthermore, the biological activity specifically referred to is triggering the dimerization of gp130 in human cells. Note the sentence in the background of the invention at page 1, lines 23-27, which reads:

> This is due to the fact that even without the intracytoplasmic domain of gp80, sIL-6R is still capable of triggering the dimerization of gp130 in response to IL-6, which in turn mediates the subsequent IL-6-specific signal transduction and biological effects (Murakami et al., 1993).

See also the simple assay of example 7. Accordingly, it would not require undue experimentation in order to determine that each analog which has 94% identity to the natural segment retains this specified activity. Reconsideration and withdrawal of this rejection is also respectfully urged.

Claims 1 and 2 have been rejected under 35 U.S.C. 102(a) as being anticipated by Fischer. The examiner states that the fusion product of Fischer is glycosylated in a similar manner to the glycosylation of naturally occurring

sIL-6R and IL-6 as it is expressed in yeast and is "essentially all" of naturally occurring sIL-6R and IL-6 and also meets the definition of "analog" absent evidence to the contrary. This rejection is respectfully traversed.

Claim 38 now specifies in paragraph (a) that the amino acid sequence is a fusion protein of the naturally occurring form of sIL-6R, including the Ig-like domain and the receptor pre-membrane region, and the naturally occurring form of IL-6. The experimental protocol on page 145 of Fischer makes clear that the sIL-6R cDNA used in the cassette only corresponds to amino acids 113-323 of sIL-6R. See also Figure 1 on page 142. As can be seen from Figure 3 of the present specification, this excludes the Ig-like domain and the receptor pre-membrane domain. Note also the paragraph bridging pages 2 and 3 of the present specification which discusses Fischer and points out that the IL-6R part of the Fischer fusion protein includes the IL-6R cytokine receptor N-domain and the cytokine receptor C-domain, but lacks essentially all of the IL-6R Ig-like domain and the receptor pre-membrane region. Clearly, therefore, this structure does not anticipate the sequence of claim 38(a). With respect to the analog of claim 38(b), this requires a maximum of 30 amino acids differences. Clearly, the sIL-6R cDNA misses the amino acids 1-112 which is the Ig-like domain and is thus much

smaller than that presently claimed and differs by many more than 30 amino acids. It also misses residues 324-356. Accordingly, as presently written, it is very clear that claim 38 is not anticipated by Fischer.

Furthermore, reference is made to example 9 of the present specification. Note particularly the paragraph beginning at page 42, line 16, which shows that the chimera which is missing the immunoglobulin-like domain of IL-6R bound to gp130 to an extent of only about 30% of other IL-6R/IL-6 chimera and is in line with the lower activity on the melanoma cell growth. Accordingly, the chimera of the present invention have superior activity and would not be obvious from anything disclosed in Fischer. Reconsideration and withdrawal of this rejection is also respectfully urged.

It is submitted that all the claims now present in the case clearly define over the references of record and fully comply with 35 U.S.C. 112. Reconsideration and allowance are therefore earnestly solicited.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made". Respectfully submitted,

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Version with Markings to Show Changes Made

In the specification:

Paragraph beginning at line 19 of page 12 has been amended as follows:

Figure 1 (A, B, C) depicts a schematic representation of the various vectors, reagents and process steps used in the construction of the chimeric DNA molecule encoding a chimeric protein in which is conserved the structure of the natural form of sIL-6R ending at the Val 356 residue followed by the sequence of the natural, mature, processed form of IL-6 as detailed in Example 1. In Fig. 1A, the ~~reverse primer is~~sequence after "*PCR with forward ... complementary to" is SEQ ID NO:9 and in Fig. 1B, the sequence after "Processed IL-6 cDNA in E.coli expression vector" is ~~EcoRI enzyme recognition site and the strand of the IL-6 cDNA sequence is presented as~~ SEQ ID NO:10.

In the claims:

Claims 1, 8, 12-15, 27-32, and 34-36 have been deleted.

Claims 2-7, 9-11, 16, 17, 20, 22-25, and 33 have been amended as follows:

2 (Amended). A chimeric sIL-6R/IL-6 ~~protein and biologically active analogs thereof~~ according to claim ~~1~~38,

wherein, in said sequence of (a), said sIL-6R is fused to IL-6 via a peptide linker molecule.

3 (Amended). A chimeric sIL-6R/IL-6 ~~protein and biologically active analogs thereof~~ according to claim 2, wherein said linker is a very short, non-immunogenic linker of about 3 amino acid residues.

4 (Amended). A chimeric sIL-6R/IL-6 ~~protein and biologically active analogs thereof~~ according to claim 3, wherein said linker is a tripeptide of the sequence ~~E-F-M (~~Glu-Phe-Met~~)~~.

5 (Twice-amended). A chimeric sIL-6R/IL-6 ~~protein and biologically active analogs thereof~~ according to claim 2, wherein said linker is a peptide of 13 amino acid residues of sequence ~~E-F-G-A-G-L-V-L-G-G-Q-F-M (~~Glu-Phe-Gly-Ala-Gly-Leu-Val-Leu-Gly-Gly-Gln-Phe-Met~~)~~ (SEQ ID NO:1).

6 (Amended). A chimeric sIL-6R/IL-6 ~~protein~~ according to claim ~~1~~ 38, being ~~the herein designated~~ sIL-6RδVal/IL-6 having a tripeptide linker of sequence ~~E-F-M~~ Glu-Phe-Met between the C-~~terminal~~ terminus ~~Val-356~~ of sIL-6R and the N-~~terminal~~ terminus ~~Pro-29~~ of IL-6, said chimeric protein having the sequence ~~set forth in Fig. 3~~ of SEQ ID NO:7.

7 (Twice-amended). A chimeric sIL-6R/IL-6 ~~protein~~ according to claim ~~1~~ 38, being the ~~herein designated~~ sIL-6RδVal/L/IL-6 of SEQ ID NO:7 in which ~~having~~ a 13 amino acid

peptide linker ~~of sequence E-F-G-A-G-L-V-L-G-G-Q-F-M~~ ~~Glu-Phe-Gly-Ala-Gly-Leu-Val-Leu-Gly-Gly-Gln-Phe-Met~~ (of SEQ ID NO:1~~)~~ is substituted for the Glu-Phe-Met of residues 357-359 of SEQ ID NO:7~~between the C-terminal Val-356 of sIL-6R and the N-terminal Pro-29 of IL-6R, said chimeric protein having the sequence set forth in Fig. 3 wherein the tripeptide of sequence E-F-M between positions 357-359 of Fig. 3 is replaced by said 13 amino acid peptide sequence~~.

9 (Amended). A chimeric sIL-6R/IL-6 ~~protein~~ according to claim ~~1~~ 38, wherein said ~~protein~~ sIL-6R/IL-6 is produced in mammalian cells ~~in a fully processed form~~.

10 (Amended). A chimeric sIL-6R/IL-6 protein according to claim 9, wherein said ~~protein~~ sIL-6R/IL-6 is produced in human cells.

11 (Amended). A chimeric sIL-6R/IL-6 ~~protein~~ according to claim 9, wherein said ~~protein~~ sIL-6R/IL-6 is produced in CHO cells.

16 (Amended). A DNA sequence encoding a chimeric sIL-6R/IL-6 ~~protein and biologically active analogs thereof~~ according to claim ~~1~~ 38.

17 (Amended). A DNA vector comprising a DNA sequence encoding a chimeric sIL-6R/IL-6 ~~protein and biologically active analogs thereof~~ according to claim ~~1~~ 38,

said vector being suitable for expression of said chimeric ~~protein~~ sIL-6R/IL-6 in mammalian cells.

20 (Amended). A DNA vector according to claim 17-~~19~~, wherein when said vector is expressed in mammalian or human cells, the expressed chimeric ~~protein~~ sIL-6R/IL-6 has a sequence that permits full processing of the chimeric ~~protein~~ sIL-6R/IL-6 by the mammalian or human cells and secretion of the fully processed chimeric ~~protein~~ sIL-6R/IL-6 from the cells into the culture medium in which said cells are grown.

22 (Amended). A DNA vector according to claim 17, wherein said vector is the ~~herein designated~~ plasmid pcDNA sIL-6R/L/IL-6 comprising a pcDNA3 vector containing the DNA sequence encoding the chimeric sIL-6R/IL-6 ~~protein~~ under the control of a cytomegalovirus (CMV) promoter, and wherein in said DNA sequence encoding said chimeric sIL-6R/IL-6 ~~protein~~ there is inserted a linker sequence encoding a peptide linker at the EcoRI site placed between the sequences encoding the sIL-6R part and the sequence encoding the IL-6 part of the protein.

23 (Amended). Transformed mammalian cells containing a DNA vector according to claim 17 which are capable of expressing the sIL-6R/IL-6 ~~chimeric protein~~ sequence carried by said vector and of fully processing the

expressed ~~protein~~ sIL-6R/IL-6 and secreting it into the culture medium in which said cells are grown.

24 (Amended). Transformed cells according to claim 23 wherein ~~in~~ said cells are ~~the herein described~~ human embryonal kidney cells 293 (HEK293) transfected by the pcDNA sIL-6R/IL-6 vector, said cells being capable of expressing the sIL-6R/IL-6 ~~chimeric protein~~, fully processing said ~~protein~~ sIL-6R/IL-6 and secreting said ~~protein~~ sIL-6R/IL-6 into the culture medium in which said cells are grown in the form of an about 85 kDa glycoprotein.

25 (Amended). A method for producing a chimeric sIL-6R/IL-6 ~~protein or biologically active analogs thereof according to any one of claims 1-14~~, comprising growing transformed cells according to claim 23 ~~or 24~~ under conditions suitable for expression, processing and secretion of said ~~protein or analogs~~ sIL-6R/IL-6 into the culture medium in which said cells are grown; and purifying said ~~protein or analogs~~ sIL-6R/IL-6 from said culture medium.

33 (Amended). A pharmaceutical composition comprising as active ingredient a chimeric sIL-6R/IL-6 ~~protein or analog thereof~~ according to claim ~~1~~ 38, and a pharmaceutically acceptable carrier, diluent or excipient.

Claim 38 has been added.